Myrmecological News	23	71-79	Online Earlier, for print 2016

Survival of persecuted myrmecophiles in laboratory nests of different ant species can explain patterns of host use in the field (Hymenoptera: Formicidae)

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Abstract

Myrmecophiles or ant associates are able to penetrate and survive inside the heavily defended nests of various ant species. With the exception of some highly specialized species, many of these myrmecophiles elicit a highly aggressive response and are frequently wounded or even killed by their hosts. Many myrmecophiles also appear to strongly prefer particular host species. The factors that allow the myrmecophiles to survive in these hostile environments and cause myrmecophiles to prefer particular host species are largely unknown. The aim of the present study was to examine the impact of the presence or absence of either the preferred host (wood ants of the *Formica rufa* LINNAEUS, 1761 group) or one of several nonpreferred ant species on the long-term survival of three obligate, unspecialized beetle myrmecophiles, *Thiasophila angulata* (ERICHSON, 1837), *Lyprocorrhe anceps* (ERICHSON, 1837), and *Amidobia talpa* (HEER, 1841), and one facultative myrmecophile, the woodlouse *Porcellio scaber* LATREILLE, 1804. In addition, we tested whether host specificity was driven by the size of the ant host workers, because host specificity has previously been demonstrated to be inversely related to aggression towards macroparasites. Our results show that despite regular aggressive host interactions, survival of the obligate myrmecophilous beetles over a period of 20 days was not different from a control set-up without ants. By contrast, the facultative ant associate *P. scaber* hardly provoked any aggressive host response, but its survival was lower in presence of *F. rufa* workers compared with a control set-up without ants. Furthermore, the data on survival in presence of nine different ant host species show that the three obligate myrmecophile beetles survived better in presence of larger-bodied ant species, and that their survival was highest in presence of their preferred host *F. rufa*, which also has relatively large workers. The only exception to this trend was the low survival observed in presence of the large-bodied ant *Camponotus vagus* (SCOPOLI, 1763). Finally, species that were less successful in killing the beetles in our tests are also shown to support more myrmecophilous rove beetles in nature. Overall, our results shed new light on the interaction between ants and various associated macroparasites and on the factors that drive observed host preferences.

Key words: Ant guests, associates, aggression, parasitism, facultative myrmecophile, obligate myrmecophile, diversity, inquiline.

Myrmecol. News 23: 71-79
ISSN 1994-4136 (print), ISSN 1997-3500 (online)

Received 7 December 2015; revision received 29 February 2016; accepted 4 March 2016
Subject Editor: Christian Rabeling

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Introduction

Parasites have an intricate relationship with their host on which they can impose substantial costs (POULIN 2011). However, hosts have evolved an array of defence strategies at the behavioural, immunological and chemical level to counter parasites (HART 1990, CLAYTON & MOORE 1997, SCHMID-HEMPEL 2011). A particularly useful system to test host-parasite interactions can be found in the nests of social insects. Social insect nests harbour a rich diversity of strictly associated symbionts including mutualists, commensals, and parasites (KISTNER 1979, HÖLLDOBLER & WILSON 1990, RETTENMEYER & al. 2011). The parasites can have a dramatic effect on host fitness by consuming brood and host resources and inducing queen and worker mortality (HÖLLDOBLER & WILSON 1990, SHMID-HEMPEL 1998, GEISELHARDT & al. 2007, BUSCHINGER 2009, HOVESTADT & al. 2012). The main defence response of social insects to macroparasites is aggression, in which they exhibit biting, stinging, spraying defensive chemicals and chasing of the intruders (HÖLLDOBLER & WILSON 1990, AYASSE & PAXTON 2002). Some ant associates or myrmecophiles evolved a specialized biology (symphiles or true guests sensu WASMANN 1894) and employ a plethora of strategies, including advanced behaviours, morphological adaptations, special defensive or appeasement

Fig. 1: Overview of the three myrmecophile beetles with their red wood ant host: (a) *Thiasophila angulata* with *Formica polyctena*, (b) *Lyprocorrhe anceps* with *Formica rufa*, and (c) *Amidobia talpa* with *Formica polyctena*. The myrmecophilous spider *Thyreosthenius biovatus* can also be observed in the centre of b.

glands and chemical mimicry. Such adaptations might lower ant aggression and enable the myrmecophiles to successfully integrate in ant colonies. What is more, they are treated as true colony members as they are fed, groomed and transported by the ants (HÖLLDOBLER & WILSON 1990, AKINO 2008, BLOMQUIST & BAGNÈRES 2010, KRONAUER & PIERCE 2011). However, some myrmecophiles are seemingly unspecialized (synechthrans, i.e., indifferently tolerated guests, and synoeketes, i.e., hostile persecuted guests, sensu WASMANN 1894): They are very similar to their non-myrmecophilous counterparts and lack the aforementioned variety of adaptations (DONISTHORPE 1927, HÖLLDOBLER & WILSON 1990). These myrmecophiles might be exposed to frequent ant aggression (DONISTHORPE 1927, PARMENTIER & al. 2015a), which can lead to an elevated stress response in the myrmecophiles, injuries and ultimately death (HÖLLDOBLER & al. 1981, NELSON & JACKSON 2009; T. Parmentier, W. Dekoninck & T. Wenseleers, unpubl.).

It is surprising how these myrmecophiles succeed to live in association with their host in such a hostile and stressful environment. The long-term effects of the host's defence response for those unspecialized myrmecophiles are unknown. Therefore, the effect of the association with host ants on the survival of three unspecialized, parasitic and myrmecophilous rove beetles associated with European red wood ants (*Formica rufa* group) was examined. First, 20-day survival of those myrmecophiles with the preferred host against survival in a control set-up without host workers was tested. The same tests were also done for a facultative myrmecophile (a species that is regularly found in ant nests, but is mainly found not to be associated with ants) to look whether the effect of host ants is similar on them compared with unspecialized myrmecophiles.

Surprisingly, many unspecialized myrmecophiles are associated with only a small group of ants (DONISTHORPE 1927, PÄIVINEN & al. 2002, PARMENTIER & al. 2014). The myrmecophiles of this study are restricted to mound building *Formica* ants. It is unclear why these relatively unspecialized myrmecophiles are only associated with mound building *Formica* species. Hitherto, it is unknown which mechanisms constrain the distribution of these species. A recent study showed that smaller workers in polymorphic red wood ant colonies are more aggressive and more successful in deterring intranidal myrmecophiles (PARMENTIER & al. 2015a). Consequently, the hypothesis under investigation is that ant species with on average smaller workers are more efficient in deterring unspecialized myrmecophiles. Interestingly, the mound building *Formica* ants have on average relatively large workers compared with other ant species in Europe (SEIFERT 2007) and support many unspecialized myrmecophiles (PARMENTIER & al. 2014). The relatively large mean worker size of these ants compared with other ant species in Europe could play a role in the strict association of many of those *Formica* associates. For that reason, we also assessed the survival of the three myrmecophilous beetle species in nests of eight other ant species spanning a gradient from one of the smallest to the largest ant species in the study area. We hypothesized that the survival rate of the unspecialized myrmecophiles would be highest in species with relatively large workers and would decrease in colonies of smaller ant species.

Materials and methods

Study species: We collected adults of three myrmecophilous rove beetles (Staphylinidae, Aleocharinae): *Thiasophila angulata* (ERICHSON, 1837), *Lyprocorrhe anceps* (ERICHSON, 1837), and *Amidobia talpa* (HEER, 1841) in European red wood ant (*Formica rufa* group) nests in populations in Northern Belgium and in Northern France during the summer and autumn of 2014 and spring and summer of 2015 (Fig. 1). Two populations (West-Vleteren, Boeschepe) consisted of *F. rufa* LINNAEUS, 1761 mounds, three of *Formica polyctena* FÖRSTER, 1850 (Beernem, Roksem, Aartrijke) mounds and both species occur sympatrically in the two remaining populations (De Haan and Beisbroek) (map see PARMENTIER & al. 2015b). Beetles were identified following FREUDE & al. (1974). We isolated the myrmecophiles by spreading nest material of *F. rufa* or *F. polyctena* nests on a large tray in the field. Ants and their brood were gently put back in the nests afterwards. DONISTHORPE (1927) categorized the three beetle species following the classification of Erich Wasmann as synoeketes, which means that the beetles are rather unspecialized in morphology and behaviour compared with advanced myr-

mecophiles (symphiles) (WASMANN 1894). Synoeketes are not treated as colony members, but mostly ignored by the ants due to their small size and behaviour (WASMANN 1894). However, we found that the three species are detected by the ants and elicited aggression (PARMENTIER & al. 2015a, supplementary videos, Appendix S1: *A. talpa*, Appendix S2: *L. anceps*, and Appendix S3: *T. angulata*, as digital supplementary material to this article, at the journal's web pages). Therefore they should rather be categorized in the group of synechtrans (unspecialized associates which provoke aggression). The complete life cycle of the beetles probably takes place inside the wood ant mounds (DONISTHORPE 1927). This was supported by the occurrence of the adults in all seasons and the recording of larvae of different stages of the beetles inside the mound from spring to autumn (and raised in the lab to adults for identification). The larvae are free-living scavengers and are not nursed or carried by the workers (T. Parmentier, W. Dekoninck & T. Wenseleers, unpubl.) in contrast with specialized beetle larvae such as *Lomechusa* and *Lomechusoides* (see HÖLLDOBLER & WILSON 1990). The larvae of *T. angulata* are very similar to non-myrmecophilous larvae of the Aleocharinae and can be reared in absence of ants (ZAGAJA & al. 2014; M. Zagaja, unpubl.). The adults are both brood predators and kleptoparasites as they prey on ant brood and food brought to the nest (PARMENTIER & al. 2015a, in press). The three beetle species can be found throughout the nest mound (edge and centre of the nest). *Lyprocorrhe anceps* and *A. talpa* have no nest location preference, whereas *T. angulata* is attracted to the densely crowded brood chambers (PARMENTIER & al. 2016). Morphological adaptations found in specialized myrmecophiles such as appeasement glands with trichomes are lacking in the three beetles (FREUDE & al. 1974). FREUDE & al. (1974) only report that the segments of the antennae of *T. angulata* are slightly compressed which could make it more difficult for ants to grab them. Their behaviour is also very similar to non-ant associated rove beetles. They escape from ant aggression by fleeing, hiding or bending their abdomen (DONISTHORPE 1927, PARMENTIER & al. 2015a; Appendices S1 - S3). They probably excrete chemicals from their bent abdomen, which is a general defence strategy of rove beetles (HUTH & DETTNER 1990). The three beetles have a similar aleocharine morphology, but differ in size (*T. angulata* mean length 10 individuals ± SD = 2.85 mm ± 0.32, *L. anceps* mean length 10 individuals ± SD = 2.16 mm ± 0.20, *A. talpa* mean length 10 individuals ± SD = 1.53 mm ± 0.10, Fig. 1). In spite of their unspecialized myrmecophilous biology, they are very specialized in their host use. Their distribution is mainly restricted to European red wood ants (*F. rufa* group) (DONISTHORPE 1927, FREUDE & al. 1974, PARMENTIER & al. 2014). There are also some records for all three species in related mound building *Formica* species. The three species were occasionally observed in nests of *Lasius fuliginosus* (LATREILLE, 1798) and there is a single record of *T. angulata* in *Lasius brunneus* (LATREILLE, 1798) (see references in PARMENTIER & al. 2014), but these are probably infrequently used hosts (T. Parmentier, W. Dekoninck & T. Wenseleers, unpubl.). The three beetles are obligate myrmecophiles, as they cannot be found away from ants. However, a large number of species can occasionally be associated with ants (DONISTHORPE 1927, ROBINSON & ROBINSON 2013, PARMENTIER & al. 2014). The widespread isopod *Porcellio scaber* LATREILLE, 1804 (adult size: 9 - 13.5 mm, BERG & WIJNHOVEN 1997, identified following BERG & WIJNHOVEN 1997) lives in a wide variety of habitats without ants (BERG & WIJNHOVEN 1997), but can also be very abundant in red wood ant mounds throughout the whole year (ROBINSON & ROBINSON 2013, PARMENTIER & al. 2014). Gravid females and juveniles were regularly observed in the mounds, which indicates that *P. scaber* is able to reproduce in the mounds. Isopods were collected in the same way as myrmecophilous beetles in red wood ant nests during spring 2015.

Ant aggression towards tested species: First, the interaction of *Formica rufa* with the three myrmecophilous beetle species and *Porcellio scaber* was examined. Therefore, a small rectangular plastic arena (length: 8 cm, width: 5.5 cm, height: 5 cm) was filled with ca. 1 cm plaster of Paris and coated with fluon. Forty *F. rufa* workers (West-Vleteren population) were acclimatized for one hour to the arena and then a myrmecophile found in the same colony was added. Ten seconds after the myrmecophile was introduced, the first twenty interactions with the ants were scored. In spite of these relatively short settling times, ants and myrmecophiles interacted similarly as in conditions where myrmecophiles were already integrated for days in lab nests (T. Parmentier, unpubl.). We also provide data of the effect of longer settling time (one hour) on ant aggression towards seven *Thiasophila angulata* beetles and compare these with the 10 s settling times (Appendix S4). These data confirm that longer settling times had no significant effect on the interaction between ants and myrmecophiles. Following interactions were observed from the perspective of the ant: ignoring (a worker's behaviour did not change when her antenna crossed the myrmecophile), showing interest (a worker started to antennate, turned her head or stopped walking or grooming when her antenna crossed the myrmecophile), opening mandibles (a worker aggressively opened her mandibles when her antennae crosses the myrmecophile), biting (a worker snapped with its mandibles and tried to grasp a myrmecophile) and acid spraying (a worker bent her gaster and sprayed acid after her antenna crossed the myrmecophile). Biting and acid spraying often followed directly after opening mandibles. In these cases only the last interaction was recorded. Ant aggression was scored by the proportion of aggressive interactions (acid spraying, biting, opening mandibles) out of the first 20 interactions. From the perspective of the myrmecophiles, the number out of 20 interactions that were directly preceded or followed by abdomen bending were counted. Trials were performed in darkness under red light and were recorded with a video camera (SONY HDR-XR550VE). Videos were subsequently analysed in VirtualDub 1.10.4 (http://www.virtualdub.org) which allowed to watch videos frame by frame.

Survival experiment: In this experiment, 20-day survival of the three beetle species in *Formica rufa* nests were compared with their survival in a control set-up without ants. *Formica rufa* workers were collected in a highly polydomous population in Boeschepe, Northern France. In addition survival of the three beetle species in nests of other ant species, ranging from one of the smallest to the largest ant species in the study region, were tested. Therefore colony fragments of *Solenopsis fugax* (LATREILLE,

1798) (Eastern bank river Meuse, Dinant), *Tetramorium caespitum* (LINNAEUS, 1758) (Duinbossen, Lombardsijde), *Lasius niger* (LINNAEUS, 1758) (urban region, Oostende), *Myrmica ruginodis* NYLANDER, 1846 (St-Sixtusbossen, West-Vleteren), *Formica cunicularia* LATREILLE, 1798 (Duinbossen, Lombardsijde), and *Lasius fuliginosus* (Provinciedomein, Raversijde; Aartrijksesteenweg, Aartrijke) were collected in different sites across Belgium during the summer and autumn of 2014 and spring and summer of 2015. Survival was also tested with *Monomorium pharaonis* (LINNAEUS, 1758) and *Camponotus vagus* (SCOPOLI, 1763) of which we already had established lab colonies. *Monomorium pharaonis* is an indoor pest in Belgium and does not occur outside buildings (DEKONINCK & al. 2003). Several colonies of *C. vagus* have only recently established in Belgium and are able to persist outdoors (DEKONINCK & PAULY 2002; W. Dekoninck, unpubl.). Ants were identified using the key provided in SEIFERT (2007).

Within one day after collecting the myrmecophiles, between 9 and 13 individuals of each rove beetle species were placed together in 1 L plastic, cylindrical containers (diameter: 8.5 cm, height: 13.5 cm) with a 1.5 - 2 cm bottom of plaster of Paris. The top 5 cm inner wall of the containers were coated with fluon to prevent ants and myrmecophiles from escaping through 20 ventilation pin holes made in the container's lid. Myrmecophiles were collected in different red wood ant populations (*Formica polyctena* and *Formica rufa*) across West Flanders, Belgium and in Boeschepe, France to obtain sufficient numbers of individuals (Appendix S5: Tab.). The tested myrmecophiles do not closely resemble the cuticular hydrocarbon profile of their red wood ant host colony (T. Parmentier, W. Dekoninck & T. Wenseleers, unpubl.). Moreover, conspecific beetles associated with *F. polyctena or F. rufa* do not substantially differ in their cuticular chemical profile (T. Parmentier, W. Dekoninck & T. Wenseleers, unpubl.). This lack of chemical adaptation to their host is further confirmed by aggression tests (PARMENTIER & al. 2016; Additional file: Tab. S3). In these tests, we compared aggression of *F. rufa* workers of one colony (West-Vleteren, description see PARMENTIER & al. 2015b) towards myrmecophiles collected in the same colony with their aggression towards myrmecophiles found in *F. polyctena* colonies. Interestingly, the aggression response of the *F. rufa* workers was not significantly different towards beetles collected in *F. rufa* or *F. polyctena* colonies. Based on these chemical and behavioural data, we argue that the myrmecophile's colony of origin did not significantly affect the results of the survival experiments. Another confounding factor that might influence myrmecophile survival in our experiments is intra- and interspecific competition. However, no aggression between the beetles was observed. By providing food ad libitum, negative competition effects on survival were minimalized. Depending on the treatment, 100 workers of either *F. rufa*, *F. cunicularia*, *Lasius fuliginosus*, *L. niger*, *Myrmica ruginodis*, *Tetramorium caespitum*, *Monomorium pharaonis*, or *Solenopsis fugax* were added. Because of their large size, only 50 workers of *Camponotus vagus* were used (cf. Fig. 3). For polymorphic species workers of all worker subcastes were used (Fig. 3). Workers were randomly picked from nests, hence we assume that all worker subcastes (or size cohorts) are represented in numbers similar to their natural distribution. In addition, between 9 and 13 individuals per beetle species were added to containers described as above, but without adding ants. These containers served as controls. Survival of myrmecophiles was monitored every two days for a total period of 20 days. Two cut maggots (larvae of *Phaenicia sericata*), an Eppendorf tube (1.5 mL) filled with water and one with honey water were provided. The same food sources were offered in the same quantities in the control containers. Eppendorf tubes were sealed with a cotton plug soaked in either water or honey water. Maggots were replaced every two days, honey water every four days. Dead ant workers were replaced by new workers of the corresponding stock colonies every two days. Corpses of myrmecophiles were also removed to prevent contamination. The containers were kept in constant dark and at room temperature (20°C ± 2°C). Every treatment was replicated between eight and ten times (Appendix S5: Tab.) with workers of another colony, except for *S. fugax*, *M. pharaonis* and *C. vagus* where we only had one colony at our disposal. For these species, different workers per replicate were used, but from the same (super)colony. Nest material of ant nests was not added to the containers. However, myrmecophiles were able to hide under dead ants, prey, Eppendorf tubes and cotton made loose by the ants.

Similarly, survival of the facultative nest-inhabitant *Porcellio scaber* was evaluated in *Formica rufa* nests and in a control set-up. Thirty specimens were monitored for 20 days in the plastic containers described above. Individuals were counted every four days. The treatment group with 100 *F. rufa* workers and the control were compared and replicated eight times (in total $8 \times 30 = 240$ individuals were tested per treatment). Water and honey water as well as two slices of carrot were provided. The latter were replaced every four days. Dead isopods were removed and dead workers were replaced every four days. For this facultative myrmecophile we also compared survival in containers with addition of 25 mL nest material in an additional experiment. We were interested whether we would observe the same effect of the ants on the isopods with much more hiding places in the nests. Nest material was taken of a deserted *F. rufa* nest and was replaced after 10 days. Here, we only counted survivors after 20 days in a treatment with 100 *F. rufa* workers and a in control treatment without workers (in total $9 \times 30 = 270$ individuals were tested with *F. rufa* and $8 \times 30 = 240$ were tested in the control treatment).

Worker size: Maximum head width of the ant species used in the survival experiment was measured. This allows us to link the mean worker size of ant species with their efficiency in killing the myrmecophilous beetles. For each ant species, maximum head width from a random set of workers was measured. More workers were measured for a given species when it showed a high degree of polymorphism ($N = 30$ for *Solenopsis fugax*, *Tetramorium caespitum*, *Monomorium pharaonis*, and *Myrmica ruginodis*, $N = 50$ for *Formica cunicularia* and *Lasius niger*, $N = 100$ for *Formica rufa* and *Camponotus vagus*).

Data analysis: All tests were two-tailed and a significance level of $\alpha = 0.05$ was used. The proportion of aggressive interactions towards the four associates were modelled with a quasibinomial GLM (family = quasibinomial in function glm) to account for overdispersion and tested with a likelihood ratio test. Subsequently, a set of quasi-

Tab. 1: Interactions between ant and associates are categorized in different categories. Mean proportions of a particular category out of a total of 20 interactions are given. 95% confidence intervals were calculated by running quasibinomial models for every interaction and with the function confint in R. They are listed in brackets under the means. Aggressive interactions are opening mandibles, biting and acid spraying. Species with a different letter code elicit a significant different proportion of aggressive interactions (Bonferroni corrected pairwise tests). The category "Abdomen bending" refers to the proportions of the 20 interactions that were directly preceded or followed by abdomen bending.

	N	**Ignoring**	**Showing interest**	**Opening mandibles**	**Biting**	**Acid spraying**	**Proportion aggressive interactions**		**Abdomen bending**
Thiasophila angulata	35	0.40 [0.34 - 0.46]	0.15 [0.11 - 0.18]	0.32 [0.27 - 0.37]	0.12 [0.08 - 0.16]	0.01 [0.01 - 0.02]	**0.45** [0.40 - 0.51]	a	0.13 [0.09 - 0.18]
Lyprocorrhe anceps	21	0.65 [0.58 - 0.72]	0.10 [0.07 - 0.15]	0.19 [0.14 - 0.24]	0.06 [0.03 - 0.10]	0.00	**0.25** [0.19 - 0.31]	b	0.15 [0.10 - 0.22]
Amidobia talpa	22	0.79 [0.72 - 0.84]	0.09 [0.06 - 0.13]	0.09 [0.06 - 0.13]	0.03 [0.01 - 0.06]	0.00	**0.12** [0.08 - 0.17]	c	0.03 [0.01 - 0.06]
Porcellio scaber	10	0.84 [0.75 - 0.91]	0.10 [0.05 - 0.16]	0.07 [0.03 - 0.12]	0.00	0.00	**0.07** [0.03 - 0.13]	c	–

binomial GLMs were conducted to compare post hoc the proportion of aggressive interactions between the four associates. P-values of these six pairwise tests were Bonferroni corrected.

In the survival analyses, survival of the three obligate myrmecophilous beetles subjected to 10 different treatments was evaluated. In particular, the survival per beetle species in nests of *Formica rufa*, in nests of eight other ant species and in a control set-up were tested against each other. Survival data per myrmecophile species were fitted with a mixed-effects Cox proportional-hazards model (THERNEAU 2012) by using the coxme function implemented in R version 3.2.1 (R DEVELOPMENT CORE TEAM 2012). This package allows the incorporation of random factors (THERNEAU 2012). In the Cox proportional-hazards model, we test whether the hazard ratio of a treatment is significantly different from 1 (COX 1972). The hazard ratio can be interpreted in our experiment as the mortality rate in a particular treatment relative to the mortality rate of a reference treatment. Treatment (i.e., ant species and control) was used as a fixed factor, replicate was modelled as a random factor. In a series of pairwise tests, survival of myrmecophiles in treatments with different ant species and the control set-up against survival in nests of *F. rufa* (reference level) was compared. P-values were estimated with a likelihood ratio test (Anova function in car package) and Bonferroni corrected. Second, survival of myrmecophiles in nests of different ant species, including *F. rufa*, against the control set-up without ants (reference level) was tested. P-values were again estimated with a likelihood ratio test and Bonferroni corrected.

For the facultative associate *Porcellio scaber*, survival data of the experiment without nest material (control vs. treatment with 100 *Formica rufa* workers), were similarly fitted with a mixed-effects Cox proportional-hazards model and significance tested with a likelihood ratio test. As we did not count *P. scaber* individuals at regular time intervals in the extra experiment with nest material, we could not do a survival analysis here. In this experiment, we only compared the proportion of surviving isopods (out of 30) after 20 days in a control set-up versus a treatment with 100 *F. rufa* workers with a quasibinomial GLM. Significance was tested with a likelihood ratio test.

Results

Aggression of *Formica rufa* towards beetles and *Porcellio scaber*: Ants exhibited frequent aggressive behaviour, such as biting, opening mandibles and acid spraying (proportions can be found in Tab. 1; Appendices S1 - S3). The proportion of aggressive interactions of *Formica rufa* towards the four myrmecophiles was significantly different (quasibinomial GLM, LR Chisq = 262.37, df = 4, P < 0.001). Bonferroni corrected pairwise tests can be found in Appendix S6: Tab. *Thiasophila angulata* elicited most aggression (proportion aggressive interactions = 0.45, CI: 0.40 - 0.51), followed by *Lyprocorrhe anceps* (proportion aggressive interactions = 0.25, CI: 0.19 - 0.31) and *Amidobia talpa* (proportion aggressive interactions = 0.12, CI: 0.08 - 0.17). When interacting with ants, beetles accelerated, turned and avoided contact. They also regularly bent their abdomen (proportion interactions in which beetles bent their abdomen: *T. angulata* = 0.13, CI: 0.09 - 0.18, *L. anceps* = 0.15, CI: 0.10 - 0.22, *A. talpa* = 0.03, CI: 0.01 - 0.06) (Tab. 1). When beetles where clamped between the ant mandibles, they always succeeded to escape. In spite of its large size, *Porcellio scaber* was largely ignored (proportion aggressive interactions = 0.07, CI: 0.03 - 0.13) and was not bitten or sprayed with formic acid during the 20 interactions of the aggression experiment (Tab. 1).

Survival of beetles and *Porcellio scaber* in nests with *Formica rufa* versus control: *Formica rufa* workers did not reduce survival in the long term for the three obligately myrmecophilous beetles compared with the control set-up (Bonferroni corrected pairwise test, *Thiasophila angulata*: *P* = 1.000, *Lyprocorrhe anceps: P* = 0.286, *Amidobia talpa P* = 1.000, Fig. 2a, b, c, Appendix S5: Tab.). Conversely, *F. rufa* workers induced a significant mortality of the facultative associate *Porcellio scaber* compared with the control set-up (Likelihood ratio test, Chisq = 7.87, P = 0.005, Fig. 2d). In an additional 20-day experiment with nest material, the proportion of surviving isopods per replicate was also significantly reduced (quasibinomial GLM, Likelihood ratio test, Chisq = 39.307, P < 0.001) in presence of *F. rufa* workers (mean = 0.85, CI: 0.78 - 0.90) compared with a control set-up without workers (mean = 0.51, CI: 0.43 - 0.59).

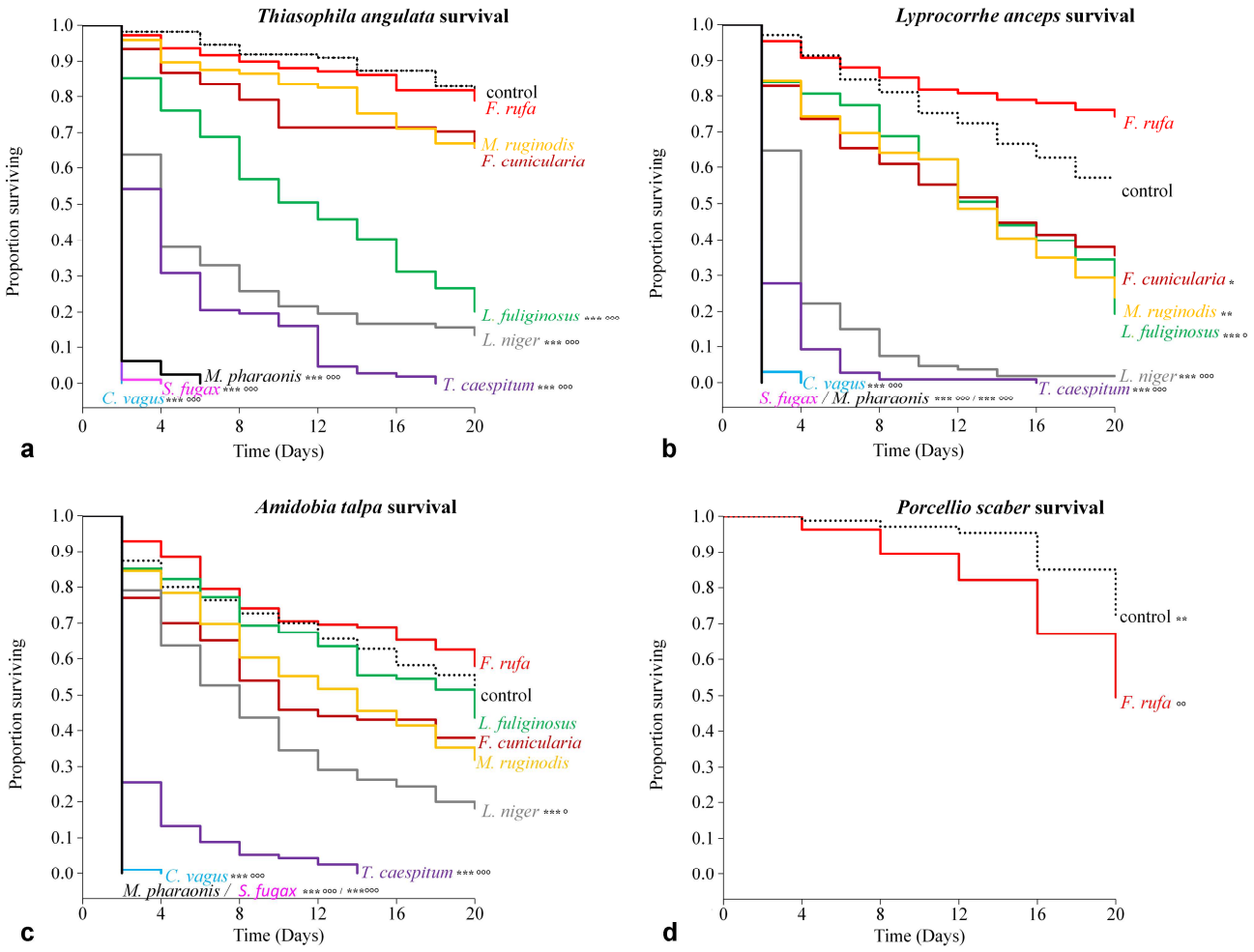

Fig. 2: Twenty day survival curves of (a) *Thiasophila angulata*, (b) *Lyprocorrhe anceps*, (c) *Amidobia talpa* and (d) *Porcellio scaber* in a treatment with the normal host *Formica rufa* and a control treatment without ants. Survival curves with other ant species are also given in a, b, and c. Significances of Bonferroni corrected pairwise tests (cf. Appendix S5: Tab.) of a treatment compared with a treatment with *F. rufa* (reference) are represented by asterisks: * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$, significances of Bonferroni corrected pairwise tests of a treatment compared with the control treatment are represented by hollow circles: ° $P < 0.05$, °° $P < 0.01$,°°° $P < 0.001$.

Survival of beetles in nests with other ant species: There was a large variation in survival of the three beetles when associated with other ant species (Fig. 2a, b, c, Appendix S5: Tab.). In general, the survival ratio of the three beetle species was very similar in nests of the different ant species. Survival of the beetles was highest when associated with *Formica rufa* workers compared with other ant species (Bonferroni corrected pairwise tests listed in Appendix S5: Tab.). *Monomorium pharaonis*, *Solenopsis fugax*, and *Camponotus vagus* killed all rove beetles (*Thiasophila angulata*, *Lyprocorrhe anceps*, *Amidobia talpa*) within the first six days, most of which did not survive the first hours. *Tetramorium caespitum* and *Lasius niger* also significantly reduced survival of all rove beetles compared with survival in *F. rufa* nests (Bonferroni corrected pairwise tests listed in Appendix S5: Tab.). *Myrmica ruginodis*, *Lasius fuliginosus*, and *Formica cunicularia* caused reduced survival in one or two beetle species compared with *F. rufa*. While there are records for the three beetle species with *L. fuliginosus* (PARMENTIER & al. 2014), survival of *L. anceps* and *T. angulata* was significantly lower when associated with this ant species compared with their preferred *F. rufa* host.

Relationship of worker size and myrmecophile survival: In Figure 3, the ln(relative mortality rate) with a particular ant species for the three beetle species vs. the maximum head width size of the ant species was plotted. The ln (relative mortality rate) of the three beetle species initially decreased linearly with larger ant species and reaches its minimum with the large *Formica rufa* species (Fig. 3). However, the extreme efficiency of the largest species *Camponotus vagus* to kill the beetles, deviates from the larger worker-higher survival pattern observed in the other eight ant species.

Discussion

Red wood ants acted aggressively towards three associated rove beetles. These obligate myrmecophiles reacted agitated, often bent their abdomen and fled away. Nevertheless, these short-term antagonistic interactions did not harm the myrmecophiles over a period of 20 days. Interestingly, survival of the common soil-dwelling isopod *Porcellio sca-*

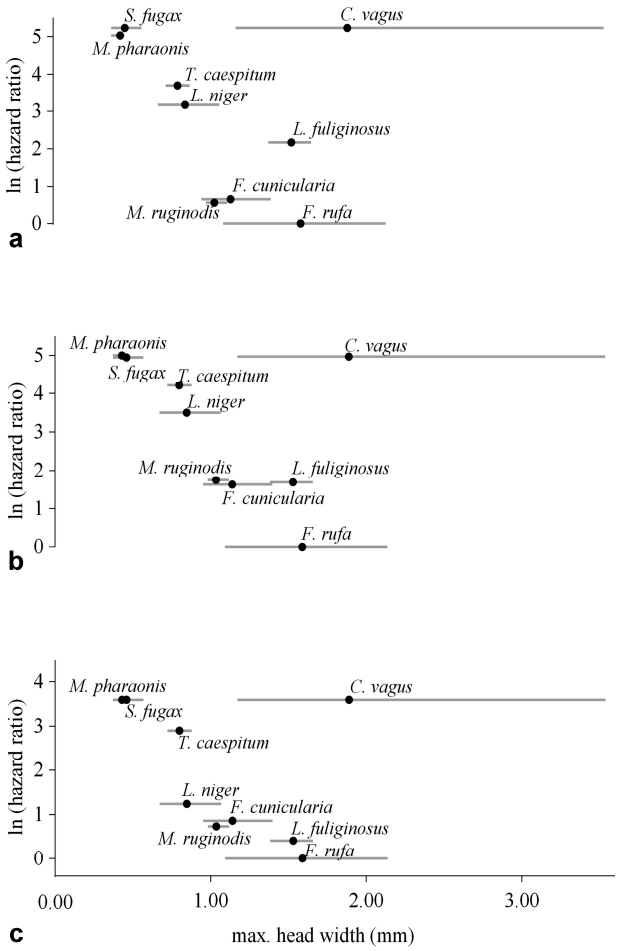

Fig. 3: Relationship between ant species size (maximum head width) and the ln (relative mortality rate) of (a) *Thiasophila angulata*, (b) *Lyprocorrhe anceps*, (c) *Amidobia talpa*. Dots show the mean of the maximum head width and grey bars the range of max head widths. Here, the reference level of the relative mortality rate is the treatment with *F. rufa*. Therefore the ln (relative mortality rate) in nests of *F. rufa* is 0 (ln (mortality rate *F. rufa* / mortality rate *F. rufa* = ln (1). = 0)).

ber, which can be highly abundant in red wood ant mounds, did decrease due to red wood ant association. Ants mostly ignored these isopods and were not observed to bite or chase them. However, isopods are reported to have a reduced life time or a lowered reproductive investment when exposed to both abiotic and biotic stress (HORNUNG & WARBURG 1994, KIGHT & NEVO 2004, CASTILLO & KIGHT 2005). The numerous interactions with ants in the experiments might indeed represent an elevated biotic stress level which ultimately led to lower survival ratios. Red wood ant mounds can still be sources rather than sinks for *P. scaber* as well as for other facultative myrmecophiles, when the benefits of a thermoregulated, moist environment with ample of food sources (ROSENGREN & al. 1987, KRONAUER & PIERCE 2011) outweigh the stress costs associated with the ants.

The three beetles have no specialized morphological (DONISTHORPE 1927, FREUDE & al. 1974), chemical (T. Parmentier, W. Dekoninck & T. Wenseleers, unpubl.) or behavioural adaptations (Donisthorpe 1927; Appendices S1 - S3) compared with more advanced myrmecophiles. We observed in all three species in varying degree, the bending of the abdomen, which stopped ants from attacking. Emitting chemicals from glands in their bent abdomen is a general defence strategy of non-ant associated and ant-associated rove beetles (HUTH & DETTNER 1990). However, it cannot be excluded that the beetles have evolved gland contents specifically adapted to deter wood ants. Possibly, the beetles have, akin to other parasitic social insect associates (FISHER & SAMPSON 1992, KILNER & LANGMORE 2011), a thicker cuticle to better resist ant bites and stings. Another possibility is that the rove beetles are difficult to catch by their small size and agility for the relatively large wood ants. The negative effects caused by ant aggression could also be compensated by indirect positive hygienic effects of the ants on the beetles. Ants possess glands which contain fungicidal and antimicrobial chemicals and these are important in suppressing pathogens in the moist and warm nests (POULSEN & al. 2002, YEK & MUELLER 2011).

In this study, we show that the general traits (fleeing, hiding, abdomen bending) of these beetles are insufficient for association with most non-host ant species. The impact of different ant species on the myrmecophiles differed dramatically and some ant species even immediately killed the beetles. It is rather surprising that red wood ants, which are commonly assumed as extremely dominant and aggressive towards other ants and arthropods (MABELIS 1979, SKINNER & WHITTAKER 1981, MABELIS 1984, BATCHELOR & BRIFFA 2010), are unsuccessful in killing or harming these beetles. Moreover, it is remarkable that the unspecialized beetles of this study only have a narrow preferred host range, i.e., mound building *Formica* ants. The relatively large size of red wood ants (SEIFERT 2007) might hamper them to successively detect, attack and / or handle small myrmecophiles and might be more suited to attack larger species, including conspecific competitors. Small animals are harder to detect and are more agile (BLANCKENHORN 2000) and size constraints can be important in explaining interactions between species. For example, large aerial insectivorous bats either cannot detect small insects, or they detect them too late to allow manoeuvring for capture (BARCLAY & BRIGHAM 1991). Therefore their diet is constrained to large and less agile insects, whereas small bats effectively detect and hunt small insects (BARCLAY & BRIGHAM 1991). Small workers in a polymorphic ant colony could have more antennal glomeruli to process olfactory cues as shown in some carpenter ants (MYSORE & al. 2009, 2010). Small workers could also be ergonomically more efficient in catching, stinging and biting myrmecophiles that match their size. Moreover, we reported recently that within a red wood ant colony, smaller workers were more aggressive than large workers towards myrmecophiles (PARMENTIER & al. 2015a). Therefore, we hypothesized that the same size-based aggression response could operate at the species level, whereby species with small workers detect and / or attack these myrmecophiles more easily and efficiently. Interestingly, survival of all three beetles indeed gradually increased with larger ant species and reached its maximum in the relatively large red wood ants (Fig. 3). However, a linear association was violated with the extreme low survival in nests of *Camponotus vagus*, the largest ant

species known for the study region. Other factors than worker size could affect the efficiency of ants to kill myrmecophiles. For example, polymorphic ant species could have size classes which are more efficient in deterring (PARMENTIER & al. 2015a) and killing myrmecophiles. In addition, the defence mechanism (acid spraying vs. stinging), the composition of defence chemicals and behaviour of ant taxa (HÖLLDOBLER & WILSON 1990) could affect the mortality rate of myrmecophiles. The observed effect on myrmecophile survival of different ant species in our tests are in line with the known diversity of rove beetle myrmecophiles associated with those ant species / taxa in Northern Europe (PÄIVINEN & al. 2002). Red wood ants have most associated myrmecophilous rove beetles (N = 26) followed by *Lasius fuliginosus* (N = 21). The subgenus *Serviformica* (includes *Formica cunicularia*) (N = 10), *Lasius* (except *L. fuliginosus*) (N = 16), and *Myrmica* species have a moderate number (N = 6). Finally, *Tetramorium* (N = 2), *Camponotus* (N = 1), *Solenopsis* (N = 0) and *Monomorium* (N = 0) have a very small or no records of associated rove beetles in Northern Europe (numbers based on PÄIVINEN & al. 2002: tab. 1). There are also no records of myrmecophilous rove beetles associated with *Solenopsis* and *Monomorium* in the European myrmecophile list of WASMANN 1894 and in the British list of DONISTHORPE (1927). It is postulated that colony size of ants (and corresponding number of niches in nests) is an important factor in predicting myrmecophile diversity (KRONAUER & PIERCE 2011). In addition to this rule, we suggest that some ant species are more successful in expelling or killing myrmecophiles, which could constrain myrmecophile distribution and host range patterns.

Acknowledgements

This project was supported by the FWOVlaanderen (Research Foundation Flanders) (grant TP no. = 11D6414N). We are grateful for the critical reviews and helpful suggestions by C. von Beeren, D. Nash and an anonymous reviewer, which substantially improved the quality of this work. We are indebted to T. Belpaeme for the English proofreading of the manuscript and M. Van Kerckvoorde for his valuable help in the field. We thank ANB (Agentschap voor Natuur en Bos) for permission to take samples.

References

AKINO, T. 2008: Chemical strategies to deal with ants: a review of mimicry, camouflage, propaganda, and phytomimesis by ants (Hymenoptera: Formicidae) and other arthropods. – Myrmecological News 11: 173-181.

AYASSE, M. & PAXTON, R.J. 2002: Brood protection in social insects. In: HILKER, M. & MEINERS, T. (Eds.): Chemoecology of insect eggs and egg deposition. – Blackwell, Berlin and Vienna, pp. 117-148.

BARCLAY, R.M.R. & BRIGHAM, R.M. 1991: Prey detection, dietary niche breadth, and body size in bats: Why are aerial insectivorous bats so small? – The American Naturalist 137: 693-703.

BATCHELOR, T.P. & BRIFFA, M. 2010: Influences on resource-holding potential during dangerous group contests between wood ants. – Animal Behaviour 80: 443-449.

BERG, M.P. & WIJNHOVEN, H. 1997: Naamlijst van de Nederlandse landpissebedden (Crustacea: Isopoda: Oniscidea). – Nederlandse Faunistische Mededelingen 7: 31-34.

BLANCKENHORN, W.U. 2000: The evolution of body size: what keeps organisms small? – The Quarterly Review of Biology 75: 385-407.

BLOMQUIST, G.J. & BAGNÈRES, A.-G. 2010: Insect hydrocarbons biology, biochemistry and chemical ecology. – Cambridge University Press, Cambridge, UK, 492 pp.

BUSCHINGER, A. 2009: Social parasitism among ants: a review (Hymenoptera: Formicidae). – Myrmecological News 12: 219-235.

CASTILLO, M. & KIGHT, S. 2005: Response of terrestrial isopods, *Armadillidium vulgare* and *Porcellio laevis* (Isopoda: Oniscidea) to the ant *Tetramorium caespitum*: morphology, behavior and reproductive success. – Invertebrate Reproduction & Development 3: 183-190.

CLAYTON, D.H. & MOORE, J. (Eds.) 1997: Host-parasite evolution: general principles and avian models. – Oxford University Press, Oxford, UK, xi + 473 pp.

COX, D.R. 1972: Regression models and life-tables. – Journal of the Royal Statistical Society, Series B (Methodological) 34: 187-220.

DEKONINCK, W. & PAULY, A. 2002: *Camponotus vagus* SCOPOLI, 1763 (Hymenoptera Formicidae) a new ant species for Belgium? – Bulletin de la Société royale belge d'Entomologie 138: 29-30.

DEKONINCK, W., VANKERKHOVEN, F. & MAELFAIT, J. 2003: Verspreidingsatlas en voorlopige Rode Lijst van de mieren van Vlaanderen. – Rapport van het Instituut voor Natuurbehoud IN.R.2003.7, 192 pp.

DONISTHORPE, H.S.J.K. 1927: The guests of British ants, their habits and life-histories. – George Routledge and Sons, London, 244 pp.

FISHER, M. & SAMPSON, B.J. 1992: Morphological specializations of the bumble bee social parasite *Psithyrus ashtoni* (CRESSON) (Hymenoptera: Apidae). – The Canadian Entomologist 124: 69-77.

FREUDE, H., HARDE, K.W. & LOHSE, G.A. 1974: Die Käfer Mitteleuropas. Band 5. Staphylinidae II. – Goecke & Evers Verlag, Krefeld, 381 pp.

GEISELHARDT, S.F., PESCHKE, K. & NAGEL, P. 2007: A review of myrmecophily in ant nest beetles (Coleoptera: Carabidae: Paussinae): linking early observations with recent findings. – Naturwissenschaften 94: 871-894.

HART, B.L. 1990: Behavioral adaptations to pathogens and parasites: five strategies. – Neuroscience and Biobehavioral Reviews 14: 273-294.

HÖLLDOBLER, B., MÖGLICH, M. & MASCHWITZ, U. 1981: Myrmecophilic relationship of *Pella* (Coleoptera: Staphylinidae) to *Lasius fuliginosus* (Hymenoptera: Formicidae). – Psyche 88: 347-374.

HÖLLDOBLER, B. & WILSON, E.O. 1990: The ants. – Harvard University Press, Cambridge, MA, 746 pp.

HORNUNG, E. & WARBURG, M.R. 1994: Oosorption and oocyte loss in a terrestrial isopod under stressful conditions. – Tissue & Cell 26: 277-284.

HOVESTADT, T., THOMAS, J.A., MITESSER, O. & ELMES, G.W. 2012: Unexpected benefit of a social parasite for a key fitness component of its ant host. – The American Naturalist 179: 110-123.

HUTH, A. & DETTNER, K. 1990: Defense chemicals from abdominal glands of 13 rove beetle species of subtribe Staphylina (Coleoptera: Staphylinidae, Staphylininae). – Journal of Chemical Ecology 16: 2691-2711.

KIGHT, S.L. & NEVO, M. 2004: Female terrestrial isopods, *Porcellio laevis* LATREILLE (Isopoda; Oniscidea) reduce brooding

duration and fecundity in response to physical stress. – Journal of the Kansas Entomological Society 77: 285-287.

KILNER, R.M. & LANGMORE, N.E. 2011: Cuckoos versus hosts in insects and birds: Adaptations, counter-adaptations and outcomes. – Biological Reviews 86: 836-852.

KISTNER, D.H. 1979: Social and evolutionary significance of social insect symbionts. – Social Insects 1: 339-411.

KRONAUER, D.J.C. & PIERCE, N.E. 2011: Myrmecophiles. – Current Biology 21: 208-209.

MABELIS, A. 1984: Aggression in wood ants (*Formica polyctena* FOERST., Hymenoptera, Formicidae). – Aggressive Behavior 10: 47-53.

MABELIS, A.A. 1979: Wood ant wars. The relationship between aggression and predation in the red wood ant (*Formica polyctena* FÖRST.). – Netherlands Journal of Zoology 29: 451-620.

MYSORE, K., SUBRAMANIAN, K.A., SARASIJ, R.C., SURESH, A., SHYAMALA, B.V., VIJAYRAGHAVAN, K. & RODRIGUES, V. 2009: Caste and sex specific olfactory glomerular organization and brain architecture in two sympatric ant species *Camponotus sericeus* and *Camponotus compressus* (FABRICIUS, 1798). – Arthropod Structure & Development 38: 485-497.

MYSORE, K., SHYAMALA, B.V. & RODRIGUES, V. 2010: Morphological and developmental analysis of peripheral antennal chemosensory sensilla and central olfactory glomeruli in worker castes of *Camponotus compressus* (FABRICIUS, 1787). – Arthropod Structure & Development 39: 310-321.

NELSON, X.J. & JACKSON, R.R. 2009: The influence of ants on the mating strategy of a myrmecophilic jumping spider (Araneae, Salticidae). – Journal of Natural History 43: 713-735.

PÄIVINEN, J., AHLROTH, P. & KAITALA, V. 2002: Ant-associated beetles of Fennoscandia and Denmark. – Entomologica Fennica 13: 20-40.

PARMENTIER, T., DEKONINCK, W. & WENSELEERS, T. 2014: A highly diverse microcosm in a hostile world: a review on the associates of red wood ants (*Formica rufa* group). – Insectes Sociaux 61: 229-237.

PARMENTIER, T., DEKONINCK, W. & WENSELEERS, T. 2015a: Context-dependent specialization in colony defence in the red wood ant *Formica rufa*. – Animal Behaviour 103: 161-167.

PARMENTIER, T., DEKONINCK, W. & WENSELEERS, T. 2015b: Metapopulation processes affecting diversity and distribution of myrmecophiles associated with red wood ants. – Basic and Applied Ecology 16: 553-562.

PARMENTIER, T., BOUILLON, S., DEKONINCK, W. & WENSELEERS, T. in press: Trophic interactions in an ant nest microcosm: a combined experimental and stable isotope (δ13C/δ15N) approach. – Oikos, DOI: 10.1111/oik.02991.

PARMENTIER, T., DEKONINCK, W. & WENSELEERS, T. 2016: Do well-integrated species of an inquiline community have a lower brood predation tendency? A test using red wood ant myrmecophiles. – BioMed Central Evolutionary Biology 16: art. 12.

POULIN, R. 2011: Evolutionary ecology of parasites (2nd edn.). – Princeton University Press, Princeton, NJ, 332 pp.

POULSEN, M., BOT, A.N.M., NIELSEN, M.G. & BOOMSMA, J.J. 2002: Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. – Behavioral Ecology and Sociobiology 52: 151-157.

R DEVELOPMENT CORE TEAM 2012: R: a language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria; <http://www.R-project.org/>, retrieved on 10 October 2015.

RETTENMEYER, C.W., RETTENMEYER, M.E., JOSEPH, J. & BERGHOFF, S.M. 2011: The largest animal association centered on one species: the army ant *Eciton burchellii* and its more than 300 associates. – Insectes Sociaux 58: 281-292.

ROBINSON N.A. & ROBINSON, E.J.H. 2013: Myrmecophiles and other invertebrate nest associates of the red wood ant *Formica rufa* (Hymenoptera: Formicidae) in north-west England. – British Journal of Entomology and Natural History 26: 67-88.

ROSENGREN, R., FORTELIUS, W., LINDSTRÖM, K. & LUTHER, A. 1987: Phenology and causation of nest heating and thermoregulation in red wood ants of *the Formica rufa* group studied in coniferous forest habitats in southern Finland. – Annales Zoologici Fennici 24: 147-155.

SCHMID-HEMPEL, P. 2011: Evolutionary parasitology: the integrated study of infections, immunology, ecology and genetics. – Oxford University Press, Oxford, UK, 516 pp.

SEIFERT, B. 2007: Die Ameisen Mittel- und Nordeuropas. – lutra Verlags- und Vertriebsgesellschaft, Tauer, 368 pp.

SKINNER, G.J. & WHITTAKER, J.B. 1981: An experimental investigation of inter-relationships between the wood-ant (*Formica rufa*) and some tree-canopy herbivores. – Journal of Animal Ecology 50: 313-326.

THERNEAU, T. 2012: coxme: Mixed effects Cox models. R package ver 2.2-3. – <http://CRAN.R-project.org/package=coxme>, retrieved on 10 October 2015.

WASMANN, E. 1894: Kritisches Verzeichnis der myrmekophilen und termitophilen Arthropoden. – F. L. Dames, Berlin, XV + 231 pp.

YEK, S.H. & MUELLER, U.G. 2011: The metapleural gland of ants. – Biological Reviews 86: 774-791.

ZAGAJA, M., STANIEC, B. & PIETRYKOWSKA-TUDRUJ, E. 2014: The first morphological description of the immature stages of *Thiasophila* KRAATZ, 1856 (Coleoptera; Staphylinidae) inhabiting ant colonies of the *Formica rufa* group. – Zootaxa 3774: 301-323.